动 物 学 研 究 1996, 17 (2): 153—167 CN 53-1040/Q ISSN 0254-5853
Zoological Research

INDUCED DEVELOPMENT OF PIGMENT MACROPHAGE AGGREGATE (PMA) IN ADULT AND JUVENILE OF THE FRESHWATER CATFISH *Silurus asotus* L.

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Abstract The development of PMA in adult and juvenile of *Silurus asotus* was investigated by peritoneally injecting with different dosages of bovine serum albumin (BSA) or Indian ink to stimulate macrophage (mφ) activity. The mφ heterogeneity was demonstrated in lymphomyeloid tissue (LT) as well as in liver with histologic and cytochemic approaches, by which 5 types of mφs, i. e. reticular / fibrous macrophage (RFmφ), shuttle macrophage (Smφ), round red macrophage (RRmφ), round yellow-red macrophage (RYRmφ) and pigment macrophage (PM), were identified. PM originated from RFmφs through the intermediate stages of Smφ, RRmφ and RYRmφ. The total amount of round macrophages (Rmφs) and PMA formation was closely related, PMA could not form if the amount of Rmφs was not high enough, and PMA had a tendency of migrating out of LT during the late experiment. The functions attributed to PMA were discussed.

Key words *Pigment macrophage aggregate, Development, Silurus asotus*

The PMA is a nodule-like structure which is composed of PM and exists in the piscine LT and liver. The pigments in PM are lipofuscin, haemosiderin and melanin (Roberts, 1975; Agius, 1985). PMA changes in number and size under circumstances such as disease (Roberts, 1975; Vogelbein *et al.*, 1987; Kranz, 1989), starvation

Abbreviation:
BSA: bovine serum albumin; GC: germinal centre; LT: lymphomyeloid tissue; MA: macrophage aggregate; mφ: macrophage; NE: non-specific esterase; PM: pigment macrophage; PMA: pigment macrophage aggregate; RFmφ: reticular / fibrious macrophage; Rmφs: round macrophages (i. e. RRmφ, RYRmφ and PM); RRmφ: round red macrophage; RYRmφ: round yellow-red macrophage; Smφ: shuttle macrophage

本文 1995 年 6 月 14 日收到, 同年 8 月 8 日修回

(Agius *et al.*, 1981) and water pollution (Wolke *et al.*, 1985; Blazer *et al.*, 1987). The amount and size of PMA are also dependent on species (Agius, 1980) and age (Agius, 1981; Brown *et al.*, 1985; Blazer *et al.*, 1987). Under experimental conditions, the peritoneally injected materials were found ultimately localized in PMA, usually accompanying the increase of PMA in size and in number. It was claimed that PMA might perform 4 kinds of functions, i.e. clearance of soluble and particulate materials from circulation, scavenging of tissue degradation products, storage of iron for reutilization, and processing antigens for immune response. Many authors further postulated that PMA should be the primitive germinal centre (GC) of teleost. However, Tsujii *et al.* (1990) claimed that PMA should be only aggregate of mφs to digest the injested materials effectively and should not be the primitive GC of teleosts.

On the development of PM (A), besides the brief description on ontogeny of PM in *Salmo* and *Tilapia* (Agius, 1981) and the induced development of PMA in liver of *Rivulus* (Vogelbein *et al.*, 1987), little data are available.

It is well known that the morphological and functional heterogeneity is very common in mammalian mφs, and at least some of these heterogeneities are the result of maturation of a single lineage of cells possessing a common precusor (Walker, 1976). The evidences on heterogeneity of teleostean mφ have also been accumulated, revealing that reticulium mφ, monocyte-mφ, RRmφ and PM exist in LT (Mesequre *et al.*, 1991). Because there are disputations on the function of PMA, and little data are available on the development of PMA, studies on the development of PMA in *S.asotus* were carried out, with particular attention to the possible relationship between PM and other mφs.

1 Materials and Methods

1.1 Fish

The adult fish, either male or female, with body weight of 120–250 g, were obtained from the market in Ji'nan, Shandong Province, and maintained in lab without food during the experiment. The water temperature was 16–22℃.

The juveniles at Day 20 post-hatch were obtained with artificial reproduction technique and raised in lab. They were feed with enough favourite food and grew well during the experiment.

1.2 Experiment design

Firstly, BSA or Indian ink were peritoneally injected into adults to stimulate mφ activities (Tab.1), and then PMA or MA (in Indian ink groups) were examined by histologic method while the other mφs were examined by cytochemic technique.

The above experiments showed that although the previousely existed PMA was rare, they still obstructed the analyse of the induced PMA, and the induced PMA was not very obvious even in the high dosage group of BSA injection (inj.). Therefore, juve-

niles at Day 20 post-hatch, which were demonstrated having no PMA in LT (authors' observations), were repeatedly injected with BSA up to 10 times (Tab.2), and then the PMA (PM) and other mφs were examined.

Table 1 Experiment designs for inducing PMA (MA) development in adults of *S. asotus* by injecting BSA or Indian ink[1]

Group No	Material injected	Dosage (mg / fish)	Sampling time (after N days of injection)		
0	— (Controls)	—	*0*	*14*	*28*
1	BSA	400	2	7	14
2	BSA[2]	1200	2	7	14
3	Indian ink	5.5	2	7	14
4	Indian ink	45	2	7	14
5	Indian ink[3]	228	2	7	14

①Two or three fish were sacrificed at each given time;

②Injected by 3 times in 3 days (200, 400 and 600 mg), and the sampling day was calculated by the last injection;

③The liver of Group 5 was sampled after 14 and 28 days of the injection

Table 2 Experiment designs for inducing PMA development in juveniles of *S. asotus* by repeatedly injecting BSA[1]

Number of injection	Dosage (ml / mg)	Accumulated dosage (mg / fish)	Age at injection (on Day N posthatch)	Age at sampling: Day N posthatch (after X days of Y-th injection)	Body weight at sampling (g)
1	0.02 / 4	4	20	22 (1: 2)	ND
2	0.04 / 8	12	23	25 (2: 2)	ND
3	0.06 / 12	24	26	—	—
4	0.10 / 20	44	29	31 (4: 2)	1.4- 2.4
5	0.14 / 28	72	32	—	—
6	0.16 / 32	104	35	37 (6: 2)	2.8
7	0.16 / 32	136	38	—	—
8	0.18 / 36	172	41	43 (8: 2)	4.0
9	0.20 / 40	212	44	—	—
10	0.22 / 44	256	47	49 (10: 2)	2.7- 4.0
		256		54 (10: 7)	5.8- 6.0
		256		61 (10: 14)	5.7- 9.2
		256		75 (10: 28)	6.0-11.0

①Two fish (4 fish on Day 61 post-hatch) were sampled at each given time;

ND: not determined.

1.3 PMA and macrophage examination

In adults of Groups 3-5, head kidney, kidney, spleen and liver were fixed in Bouin fixtative, embedded in paraffin, sectioned at 5-7 μm, stained with H.& E. In the adults of Groups 0-2 and juveniles, tissues were fixed in F-Ca fixtatives (in 10% sucrose) at 4℃ for 24 h, stored in Holt's solution (0-4℃) for 24 h, embedded in 52℃ paraffin and serially sectioned at 5-7 μm. Some sections were stained for NE to indentify mφs, the others were stained with H.& E. to examine PMA.

1.4 Statistics

The relative amount of Rmφs were examined on NE-staining sections. Ten sections were chosen at random and 1 mm^2 area of each section was chosen randomly, and the total number of Rmφs were counted. The means and standard errors were caculated.

In adults received Indian ink inj., the amount carbon-loaded Rmφs after 14d of the injs.was also examined using H.E. stained sections.

1.5 Ultrastructure of induced PM in juveniles

Juveniles after 14d of the 10th BSA inj. were sacrificed and their head kidney, kidney and spleen were examined with TEM techniques.

2 Results

2.1 The head kidney

The mφs in the head kidney may be divided into the following 5 types on the basis of NE and H.E.staining, i.e. (1) RFmφ, characterized by the long and thin cell process and being light red in NE staining; (2) Smφ, much shorter (4-6 μm in length) and broader than the fibrous mφ. It is bright red in NE staining. (3) RRmφ, the cytoplasm is eosin-philic and contains no or a few melanin granules but no brown pigment (lipofusin), it is bright red on NE stained sections; (4) RYRmφ, the cytoplasm is still eosin-stainable but contains some brown or yellow pigments, thus appeared as yellow-red on H.E. stained specimens, and in NE staining it is deep red; (5) PM, the cytoplasm contains a lot of yellow pigments and is eosin-nonstainable. In NE staining it is also strongly positive (deep red).

In adult Group 0, the PM was rare and scattered in LT, and small PMA, which was composed of several PMs, might be observed.The number of Rmφs was relatively low (Tab.3). After starved for 14d, PMA and mφs distribution was similar to that of the normal controls. After starved for 28d, RRmφ and Smφ were frequently encountered (Tab.3).

In adult Group 1, after 2d of the inj., the distribution of mφs and PMA were similar to that of the controls. After 7d of the inj., the amount of fibrous mφ, Smφ and RRmφ were relatively high, the number of Rmφs also increased, but PMA did not change obviously. After 14d of the inj., small PMAs with several or more PMs were easy to be found, and occassionally the RYRmφ might be found in PMA (Tab.3).

In adult Group 2, after 2d of the inj., PMA and PMs were similar to those of the controls, but a lot of Smφs and a few RRmφs were distributed randomly. After 7d of the inj., RRmφs, RYRmφs and PMs, all increased in number, and small PMA were easy to be found, in which RRmφs and RYRmφs might be occassionally observed. After 14d of the inj., PMA was similar to that after 7d of the inj., but RRmφs decreased in number and were not so easy to be found in PMA (Tab.3).

Table 3 Changes of mφs in LT and liver of adults of *S. asotus* after receiving BSA inj.

Tissue	Dosage (mg / fish)	Sampling time (after N days of injection)	RFmφ	Smφ	RRmφ	RYRmφ	PM	MA	PMA	Amount of total Rmφs [1]
Head		0	+	±	±	±	+	−	+	78.5 ± 19.3
kidney	0	14	+	±	±	±	+	−	+	71.4 ± 9.1
	(Controls)	28	+	+	+	±	+	±	+	77.6 ± 14.0
	400	2	+	±	±	±	+	−	+	68.7 ± 11.8
		7	+	+	+	±	+	−	+	93.2 ± 14.5
		14	+	±	+	+	+	−	+	106.4 ± 14.0
	1200	2	+	+	+	±	+	−	+	110.0 ± 19.7
		7	+	±	+	+	+	−	+	148.9 ± 13.3
		14	+	±	±	+	+	−	+	177.2 ± 22.8
Kidney	0	0	+	+	±	±	+	−	+	62.0 ± 12.4
	(Controls)	14	+	+	±	±	+	−	+	62.7 ± 7.6
		28	+	+	−	±	+	−	+	65.4 ± 13.3
	400	2	+	+	±	±	+	−	+	62.6 ± 8.6
		7	+	+	+	+	+	−	+	90.4 ± 15.4
		14	+	+	+	+	+	−	+	96.4 ± 14.0
	1200	2	+	+	+	±	+	−	+	102.9 ± 9.5
		7	+	+	+	+	+	−	+	134.8 ± 28.0
		14	+	+	+	+	+	±	+	131.8 ± 21.0
Spleen	0	0	+	+	+	±	+	−	+	109.7 ± 28.5
	(Controls)	14	+	+	+	±	+	−	+	94.0 ± 10.2
		28	+	+	+	+	+	−	+	93.9 ± 15.1
	400	2	+	+	+	±	+	−	+	92.7 ± 14.0
		7	+	+	+	±	+	−	+	94.9 ± 22.0
		14	+	+	+	+	+	−	+	114.7 ± 17.6
	1200	2	+	+	+	±	+	−	+	104.1 ± 21.1
		7	+	+	+	+	+	+	+	136.2 ± 26.7
		14	+	+	+	+	+	+	+	196.1 ± 27.4
Liver	0	0	±	−	+	±	−	−	−	14.1 ± 2.0
	(Controls)	14	±	−	+	±	−	−	−	10.7 ± 4.0
		28	±	−	+	±	±	±	−	15.4 ± 5.7
	400	2	±	−	+	±	−	−	−	9.6 ± 3.3
		7	±	±	+	+	−	±	−	14.0 ± 4.0
		14	±	±	+	+	−	±	−	12.9 ± 3.2
	1200	2	±	−	+	−	−	−	−	11.3 ± 2.8
		7	±	−	±	±	±	−	±	20.2 ± 8.5
		14	±	−	±	±	±	−	±	19.1 ± 6.4

[1] The amount of Rmφs (RRmφ, RYRmφ and PM) is expressed as "means ± standard error" / mm^2. +: observed; −: not observed; ±: observed but rare. MA is aggregate of RRmφs or RYRmφs or mixed (RRmφ, RYRmφ and PM, but PM is not dominate).

In adult Group 3, after 2d of the inj., many RFmφs which had engulfed a lot of carbon particles were found, but the carbon-laden Rmφs were rather rare. The previously existed RYRmφ, PM and PMA did not intake any carbon particle. After 7d of the inj., besides the RFmφs and a few Rmφs, a lot of carbon-laden Smφs were noted. Af-

ter 14d of the inj., the carbon-laden mφs increased in number, but they distributed at random and did not form MA. A few carbon-laden Rmφs moved into the previously existed PMA, thus formed the mixed MA with yellow and black colours.

Table 4 Induced development of MA (composed of round, carbon-laden mφ) in adult of *S. asotus* after receiving Indian ink injection

Tissue	Dosage (mg carbon / fish)	Sampling time (after N days of injection)	RFmφ ①	Smφ ①	Rmφ ①	MA ②	Amount of round, carbon-laden mφ ($M \pm S.E.$)(n / mm²)③
Head	5.5	2	+	+	±	−	
Kidney		7	+	+	+	−	
		14	+	+	+	−	24.6 ± 5.0
	45	2	+	+	±	−	
		7	+	+	+	−	
		14	+	+	+	−	144.0 ± 21.0
	228	2	+	+	+	−	
		7	+	+	+	+	
		14	+	+	+	+	8731.2 ± 959 3
Kidney	5.5	2	+	+	−	−	
		7	+	+	±	−	
		14	+	+	+	−	21.9 ± 5 7
	45	2	+	+	±	−	
		7	+	+	+	+	
		14	+	+	+	+	78.3 ± 16.4
	228	2	+	+	+	−	
		7	+	+	+	+	
		14	+	+	+	+	6436.8 ± 1344 3
Spleen	5 5	2	+	+	+	−	
		7	+	+	+	+	
		14	+	+	+	+	301.7 ± 882 5
	45	2	+	+	+	+	
		7	+	+	+	+	
		14	+	+	+	+	389.3 ± 70.2
	228	2	+	+	+	+	
		7	+	+	+	+	
		14	+	+	+	+	6016.0 ± 1487.3
Liver	5.5	2	−	−	−	−	
		7	−	−	+	−	
		14	−	−	+	−	2.6 ± 2.2
	45	2	−	−	−	−	
		7	±	±	+	−	
		14	±	±	+	−	4.5 ± 2 4
	228	14	±	+	+	−	60.7 ± 9.5
		28	±	±	+	±	61 4 ± 19 5

①*carbon-loaded*; ②*composed of round, carbon-laden mφs*; ③since carbon-loaded round mφ was not dominate and rare compared with other carbon-loaded mφs after 2 or 7 days of the injection, the amount of round carbon-loaded mφ was not examined +, −and ± are same as in Table 3.

In adult Group 4, after 2d and 7d of the inj., the distributions of carbon-laden mφs were similar to their counterparts in the Group 3. After 14d of the inj., many carbon-laden Rmφs appeared and some of them formed loosely-packed MA (Tab. 4, Fig.1).

In adult Group 5, after 2d of the inj., a lot of carbon-loaded RFmφs, Smφs and Rmφs were observed, and some of the carbon-laden Rmφs had formed small MA. After 7d of the inj., many large MAs consisting of carbon-laden Rmφs were observed (the largest with a size of 310 μm × 260 μm). The large MA usually situated near vessels or encircled the small vessels. After 14d of the inj., MA further increased in size, and some smaller MAs were observed fusing larger MA (Tab.4, Fig.2).

In juvenile group, after 2d of the 1st or 2nd injs., no PM or PMA was observed, and the total numbers of Rmφs were low (Tab.5). After 2d of the 4th BSA inj., the amount of Rmφs increased markedly, most of them were RRmφs or RYRmφs and scattered in LT, only a few of them had formed small MA (the largest size 45 μm × 26 μm). Typical PM was rare, and no PMA was observed. After 2d of the 6th and 8th injs., MAs increased both in number and in size, as the total number of Rmφs increased. PMs also raised in number, but no PMA was observed. After 2d of the 10th inj., PM increased futher and outnumbered the RRmφs and RYRmφs, and PMAs were frequently found, and the largest PMA was 50 μm × 40 μm in size. After 7d of the 10th inj. PMA increased in number and size, and gradually became compact, in which few other kinds of cells were encountered. After 14d of the 10th inj., the compact PMAs were usually found located near vessels and enclosed by a thin layer of fibrous connective tissue. MAs became rare, and the total number of Rmφs decreased. After 28d of the 10th inj., PMA usually situated near large vessels and was very compact, but the total number of Rmφs decreased further. The PM was the dominate type of Rmφ s as the RRmφs and RYRmφs reduced markedly in number and became rare (Tab. 5, Figs.3-5).

2.2 The trunk kidney

In trunk kidney, LT was scattered among renal units and collecting duct system. The mφ heterogeneity was same as that in head kidney. In adult Group 0, the distributions of PMA and other mφs were similar to their counterparts of head kidney, but the amounts were lower than those in head kidney (Tab. 3). In adult Groups 1 and 2, PMA did not change obviously during the experiment, the change of other mφ was similar to that in head kidney and was summarized in Tab.3. In adult Groups 3-5, the MA development was summarized in Tab. 4, and in Group 5, the carbon-laden Rmφs were frequently observed migrating towards the lumen of collecting ducts and mesonephric duct after 7d and 14d of the inj. (Fig.6).

After juveniles received repeated BSA injs., the induced PMA development was similar to that in head kidney, and was shown in Tab. 6.

2.3 The spleen

As shown in Tabs.3, 4 and 7, the induced PMA (MA) development was similar to that in head kidney. However, the ellipsoid mφ was a main source of forming PMA. In adult Group 3, MA was observed after 7 and 14d of the inj. (Fig.7), and the amount of carbon-laden Rmφs was much higher than those in head kidney and kidney. In adult Group 5, MAs were found migrated towards to the hilus after 14d of the inj. (Fig. 8), and the largest MA was up to 1000 μm × 300 μm in size. In juveniles received repeated BSA injs., after 2d of the 8th inj., PMA was observed migrate to the hilus. As PM (A) and RYRmφ migrated towards to the hilus, the amount of Rmφs decreased markedly after 28d of the last inj. (Tab. 7, Figs.9-11).

Table 5 Induced development of PMA in head kidney of juvenile of *S. asotus* after receiving repeated BSA injections

Sampling time: Day N post-hatch	Accumulated dosage (mg. fish)	RFmφ	Smφ	RRmφ	RYRmφ	PM	MA	PMA	Amount of Rmφs ($M \pm S.E$) (n / mm²)
Day 22	4	+	+	+	-	-	-	-	13.9 ± 9.4
Day 25	12	+	+	+	-	-	-	-	38 7 ± 7.3
Day 31	44	+	+	+	+	±	+	-	99.9 ± 22.9
Day 37	104	+	+	+	+	+	+	-	181 4 ± 35 3
Day 43	172	+	+	+	+	+	+	-	199.4 ± 33.3
Day 49	256	+	+	+	+	+	+	+	237 0 ± 50 2
Day 54	256	+	+	+	+	+	+	+	439.2 ± 55.9
Day 61	256	+	+	+	+	+	-	+	342 0 ± 62 9
Day 75	256	+	+	±	±	+	-	+	227.1 ± 49.8

Table 6 Induced development of PMA in kidney of juvenile of *S. asotus* after receiving repeated BSA injections

Sampling time: Day N post-hatch	Accumulated dosage (mg./fish)	RFmφ	Smφ	RRmφ	RYRmφ	PM	MA	PMA	Amount of Rmφs ($M \pm S.E.$) (n / mm²)
Day 22	4	+	+	+	-	-	-	-	10.2 ± 5.5
Day 25	12	+	+	+	+	-	-	-	31.0 ± 8 5
Day 31	44	-	+	+	+	+	+	-	43.2 ± 7 8
Day 37	104	+	+	+	+	+	+	-	70.9 ± 12.8
Day 43	172	+	+	+	+	+	+	-	120.7 ± 16.3
Day 49	256	+	+	+	+	+	+	+	155.2 ± 29.1
Day 54	256	+	+	+	+	+	±	+	258.2 ± 42.1
Day 61	256	+	+	+	+	+	-	+	261 0 ± 65 0
Day 75	256	+	+	±	±	+	-	+	180.9 ± 50.3

2.4 The liver

The mφs were rather poorly developed in the liver, and RFmφ, Smφ and Rmφ were scarce in the controls. After adults received BSA inj., small MA or PMA might be induced (Tab.3). In juveniles received repeated BSA injs., PMA was induced and its development was similar to those in LT, but it was rather rare and the total number of

Rmφs was very low (Tab.8). In adult Groups 3 and 4, no MA was induced during the experiment. While in adult Group 5, the carbon-laden Rmφs were found having formed small MA after 28d of the inj. (Tab.4).

Table 7 Induced development of PMA in spleen of juvenile of *S. asotus* after receiving repeated BSA injections

Sampling time: Day N post-hatch	Accumulated dosage (mg./fish)	RFmφ	Smφ	RRmφ	RYRmφ	PM	MA	PMA	Amount of Rmφs ($M \pm S.E$) (n/mm²)
Day 22	4	−	+	+	−	−	−	−	111.1 ± 30.6
Day 25	12	−	+	+	+	−	−	−	188.0 ± 61.2
Day 31	44	−	+	+	+	±	−	−	423.9 ± 73.7
Day 37	104	−	+	+	+	+	+	−	514.1 ± 90.3
Day 43	172	−	+	+	+	+	+	−	281.2 ± 43.5
Day 49	256	+	+	+	+	+	±	+	486.5 ± 192.0
Day 54	256	+	+	+	+	+	±	+	555.2 ± 191.7
Day 61	256	+	+	+	±	+	−	+	529.3 ± 146.3
Day 75	256	+	+	+	±	+	−	+	357.1 ± 99.3

Table 8 Induced development of PMA in liver of juvenile of *S. asotus* after receiving repeated BSA injections

Sampling time: Day N post-hatch	Accumulated dosage (mg./fish)	RFmφ	Smφ	RRmφ	RYRmφ	PM	MA	PMA	Amount of Rmφs ($M \pm S.E$) (n/mm²)
Day 22	4	±	−	−	−	−	−	−	0
Day 25	12	±	−	−		−	−	−	0.1 ± 0.3
Day 31	44	±	−	−	−	−	−	−	1.1 ± 1.6
Day 37	104	±	−	−	−	−	−	−	2.2 ± 1.4
Day 43	172	±	−	−	−	−	−	−	1.9 ± 1.8
Day 49	256	±	−	−	+	−	+	−	5.7 ± 2.4
Day 54	256	±	−	−	+	−	+	−	5.7 ± 2.9
Day 61	256	±	−	+	+	−	−	−	7.3 ± 4.7
Day 75	256	±	−	+	+	+	−	−	10.0 ± 7.1

2.5 MA in the mesenterum

On liver specimens of juveniles after 2d of the 10th BSA inj., and on spleen specimens of juveniles after 14d of the 10th inj., mesenterum attached to these tissues were preserved by chance. In the mesenterum, large MAs, which were composed of RYRmφs, were observed. However, they were not encircled by a thin layer of fibrous connective tissue (Fig.12).

2.6 Ultrastructure of induced PM in juvenile

After 14d of the 10th BSA inj., PM in the spleen, head kidney and trunk kidney was highly specilized. The nucleus was small and indented, the cytoplasm was replete with electron-dense body, multimembranous body, or elongate body with electron-dense crystal lattices. However, typical organills such as mitochondria, phagosomes were rare or lacking (Figs.13, 14).

3. Discussion

3.1 PMA development and the final fate of PM

As in mammals, the mφ heterogeneity also exists in teleostean LT (Mesequre *et al.*, 1991). The NE staining is one of the reliable technique for indentifying mφs (Kaplow, 1981). In this research, the mφ heterogeneity was found existing in LT of the freshwater catfish with histologic and cytochemic methods, by which 5 types of mφs were identified.

Agius (1981) briefly described the ontogenetic development of PMA (PM) in *Salmo* and *Tilapia*, but the relationship between PM and the other mφs was ignored. Vogelbein *et al.* (1987) suggested that the induced hepatic PMA was originated from monocytes in blood, and the accumulation of pigments in this structure was also recorded. The migration of mφs to PMA was found in turbot spleen (Ferguson, 1976) and kidney of sea horse (Tsujii *et al.*, 1990). In the present study, the induced PMA (MA) development in adults and especially in juveniles of the catfish shows that PM come from other m φ s, and may be summarized as follow:

RFmφ---> Smφ ---> RRmφ ---> RYRmφ ---> PM

During this process, the cell volume enlarged, the phagoctotic ability gradually decreased and finally lost, the pigments accumulated step by step, and the NE activity raised gradually. The RRmφ, RYRmφ and PM, all were capable to migrate to form MA provided the amount was high enough.

The development of PMA in head kidney, kidney and spleen was similar, except that in spleen the ellipsoid mφs were a main cell source for forming PMA (MA), and PMAs (MAs) were much more abudant than those in head kidney or kidney (Tabs.3–7).

In liver, PMA development was similar to those in LT. However, because mφs were rather poorly developed, the PMA and Rmφs were very rare. The mφ s for forming PMA seemed to originate from monocytes in blood vessel, and this is similar to the observatios of Vegelbein *et al.* (1987), but in catfish the monocytes which migrated out of blood vessels had already engulfed a lot of injected materials, and PMA development was not, as in Vogelbein *et al.* (1987) 's study, an inflammatory process.

It is well known that cell degeneration is a common phenomenon in PMA. In this study, it was found that, besides a few PMs disrupted in LT, most PMs had a tendency of migrating out of LT, and this phenomenon was especially prominent in spleen and thymus (data not shown here) of juveniles. In spleen, PMA migrated towards to the connective tissue at the hilus along the large blood vessels. In thymus, the RYRmφs and PMs moved towards to the outer zone, passed through the base membrane and thus (being) released into the gill cavity. The amounts of total Rmφs in head kidney, kidney, spleen and thymus after 28d of the 10th BSA inj. were markedly lower than

those after 7d of the 10th BSA inj. (Tabs. 5–8), and this phenomenon may be reasonablly explained with migration. In adults received Indian ink inj.with high dosage (220 mg carbon per fish), the amount of carbon-laden Rmφs was extremely high, they also showed a tendency of migrating out of LT. and in kidney, they were observed migrate into the lumen of collecting and nephric ducts and thus separated from renal LT.

The induced development of PMA, the degeneration and disrupture of PM, and the migration of PM (A), all indicated that PM should be effete mφ. In the authors' opinion, the excessive PM is harmful for maintaining the stable microenvironment of LT, and the disrupture of PM, which shall release the accumulated indigestable materials, is much more harmful to LT. Therefore, the migration of PM (A) out of LT is beneficial to maintain stable microenvironment of LT.

Tsujii *et al.* (1990) postulated that forming PMA was for digesting the injected material more effectively. However, in the present study, it was found that PMA (MA) could not form provided the total number of Rmφs was not high enough, and the PMA in juveniles was induced after a long time of repeated BSA injs.with high dosages (Tabs.5–8). Therefore, it seemed that forming PMA is responsible for limitting contact of PM with LT. This viewpoint is further supported by the fact that PMA became compact and was usually encircled by a thin layer of fibrous connective tissue during the late stage of induced experiment for PMA development in juveniles.

3.2 Effect of injection dosage on PMA (MA) formation

As mentioned in the Introduction, the variation of species, ageing, disease, starvation and water pollution, all can affect the amount and size of PMA. Under experimental conditions, the artificially injected materials, such as carbon particle, protein and bacteria, can make PMA increase in number and size. In the present study, it was found that the induced PMA (MA) is closely related to the injected dosage, i.e.the higher the dosage is, the more and larger PMA (MA) may be induced (Tabs. 3–8). Since the amount of scavaged materials by one mφ is not limitless, the amount of mφs needed to handle the injected materials increased with the increase of inj. dosage. As more mφs had been used up, the more PMA might form.

Under the same experimental conditions, however, the amount as well as the size of PMA (MA) in spleen were much more higher or larger than those in head kidney and kidney, and the amounts of Rmφs in spleen were also markedly higher than those in the latter two (Tabs.3, 4, 7). This was due to the developed mφs in spleen. Besides RFmφ, Smφ and RRmφ in splenic pulp, the ellipsoid mφs were rather developed and constituted the first defence to remove the injected matrials from blood circulation. Compared with LT, mφs in liver was rather poorly developed (Tabs.3, 4, 8). Since PMA (MA) in liver originated from monocyte in blood circulation which had engulfed the injected materials, the amount of PMA (MA) was also increased as the increase of inj. dosage.

It is very obvious that carbon particles are much more difficult for mφs to handle than BSA. In adults received 1200 mg BSA inj., the induced PMAs were not very obvious although the total number of Rmφs also increased. In adults received Indian ink inj. with 45 mg carbon per fish, a lot of MAs (composed of carbon-laden Rmφs) were induced during the experiment and the amount carbon-laden Rmφ was very high (Tabs.3, 4).

3.3 PMA and scavenger

Since the injected materials were finally found in PMA (MA) (Ellis *et al.*, 1976; Secombes *et al.*, 1980), it was postulated that PMA can remove the injected materials from circulation. However, Tsujii *et al.* (1990) found that, in the kidney of sea horse, the peritoneally injected carbon and ferritin were taken up by sinusoid mφs rather than PMA. The depleted mφ then migrated towards the originally existed PMA or formed new PMA (in early stage it is in fact the MA rather than PMA—present authors). In fact, the migration of mφ towards PMA was described much earlier (Ferguson, 1976; Ellis *et al.*, 1976).

In this study, it was found that PMA, PM and RYRmφ did not take up the injected carbon. In juveniles received repeated BSA injs., typical PMA was observed after 2d of the 10th BSA inj., revealing that clearance of BSA from circulation did not depend on PMA. Ultrastructurally, the PM was highly specialized. During the late stage of induced PMA development in juveniles, PM (A), as well as RYRmφ in thymus, was observed migrate out of LT. Therefore, as pointed out in 3.1, PM should only be a kind of effete mφ which has already lost phagocytic ability, and PMA should only be cell aggregate composed of PMs for limitting contact of PM and LT. The materials in PMA is uptaken by their precusors such as RFmφ, Smφ and RRmφ.

Because PMA increases under the conditions of disease, infection, and starvation, it was also suggested that PMA can scanvage tissue degradation products (Roberts, 1975; Agius *et al.*, 1981; Kranz, 1989). As discussed above, it is suggested that PMA can not scavage tissue degradation products. Under these circumstances, the increase of PMA is the result of vigorous activity of other mφs which has been stimulated by the tissue degradation products.

3.4 PMA and germinal centre

Because antigenic and non-antigenic materials can ultimatelly localized in PMA and PMA is nodule-like shaped, PMA was postulated as the primitive GC of teleosts (Roberts, 1975; Ferguson, 1976; Ellis *et al.*, 1976; Agius, 1985; Lammers, 1986). Agius (1985) further analysed the similarity and difference between PMA and GC. However, Tsujii *et al.* (1990) emphasized that they failed to find any mitosis figure in PMA and claimed that PMA should not be the primitive GC of teleosts.

On the basis of the present study and the data available in literature, the authors hold the viewpoint that PMA should not be regarded as the primitive GC of teleosts.

and the pyronin-philic cell cluster might be functional anlage to GC (Secombes *et al.*, 1982). Firstly, according to this study, the antigenic materials accumulated in PMA is taken up by its precusors rather than PMA. PMA is derived from the vigorous activity of other mφs, so PMA increases in size and in number after the fish receives antigenic stimulation. Immunocytochemic techniques have also revealed that antigenic material was first appeared in ellipsoid mφs (in spleen), scattered mφs (in kidney) and some fibrous-shaped cells rather than in PMA (Secombes *et al.*, 1980).

Secondly, Lammers (1986) observed small lymphocytes around PMA, while Tsujii *et al.* (1990) found no small lymphocyte near PMA and no mitosis figure in PMA. According to the induced experiment in juveniles of catfish, during early stage, PMA was loosely packed, in which PM and RYRmφ could contact directly with LT; while in late stage, PMA became compact, either migrated out of LT along large blood vessels or was enclosed by a thin layer of fibrous connective tissue. So PMA gradually lost contact with LT during the development.

Thirdly, it has been reported that PMA increases with ageing and is abundant in old fish (Agius, 1981; Brown *et al.*, 1985; Blazer *et al.*, 1987). However, the GC of higher vertebrates should not increase with ageing.

Phylogenetically, from Cyclostomata, Chondrichthyes to Osteichthyes, PMA also changes from randomly scattered PM to nodule-like structure, and this was regarded as another evidence for PMA being primitive GC (Agius, 1980, 1985). However, recent studies reveal that the immune system of teleosts is not more advanced than Chondrichthyes. From the differentiation level of LT and the diversity of immunoglobulin, the immune system of Chondrichthyes seems more differentiated than that in teleosts. Because Chondrichthyes usually live in sea (usually much less contaminated than freshwater), so their mφs seem to have fewer chances to be stimulated by water pollution via the increased tissue degradation product. In the authors' opinion, the nodule-shaped PMA is related to the relatively developed mono-nuclear phagocyte system of teleosts as a compensation for the relatively undeveloped humoral immune system.

Finally, PMA (MA) was observed in liver and mesenterum of catfish, but they are not lymphomyeloid organs.

3.5 The relation between PMA and fish disease and water pollution

PMA was hold as indicator for fish health condition (Roberts, 1975; Wolke *et al.*, 1985; Kranz, 1989) and water pollution (Wolke *et al.*, 1985; Blazer *et al.*, 1987). This study revealed that PMA derives from the activity of other mφs. It is very obvious that disease and water pollution increase, directly or indirectly, the tissue degradation product of fishes, which in return enhances the activity of mφs, and finally PMA increases. So the authors are in agreement with this viewpoint, but it should be understood in application that a period of interval time exists between the vigorous activi-

ty of mφs and PMA formation.

Figure Legends

1. Head kidney of adult after 14d of Indian ink inj. (45 mg dry materials per fish), showing the carbon-laden Rmφs had formed small and loosely packed MA (H & E, ·50).
2. Head kidney of adult after 14d of Indian ink inj (228 mg dry materials per fish), showing the carbon-laden, Rmφs had formed many large MA (H & E, ×25).
3. Head kidney ofjuvenile after 2d of the 6th BSA inj., showing Rmφs accumulated near blood vessel (NE, ×50).
4. Head kidney of juvenile after 14d of the 10th BSA inj., showing the large and compact MA near the blood vessel. The MA was encircled by a thin layer of fibrous connective tissue (NE, ×50).
5. Head kidney of juvenile same as in Fig.4, showing MA in NE staining was typical PMA (arrow) (H & E, ×50)
6. Trunk kidney of adult after 14d of the Indian ink inj. (228 mg dry materials per fish), showing carbon-laden mφs located around a large collecting duct, and some of them had already moved into the lumen (H & E, ×50).
7. Spleen of adult after 14d of the Indian ink inj. (5.5 mg dry materials per fish), showing the carbon-laden, Rmφs had formed MA These MAs encircled or situated near small blood vessels (H & E, ×25).
8. Spleen of adult after 14d of the Indian ink inj. (228 mg dry materials per fish), showing large MA (H & E, ×25).
9. Spleen of juvenile after 2d of the 4th BSA inj., showing the Rmφs had not formed typical MA yet (NE, ×100).
10. Spleen of juvenile after 2d of the 6th BSA inj., showing the Rmφs accumulated around blood vessels in the central portion of the spleen (NE, ×50).
11. Spleen of juvenile after 14d of the 10th BSA inj., showing a compact MA. It was enclosed by a very prominent fibrous connective tissue and situated near blood vessel (NE, ×100).
12. Mesenterum of juvenile after 14d of the 10th BSA inj, showing the large MA.It was composed of RYRmφs (H & E, ×100).
13. Head kidney of juvenile after 14d of the 10th BSA inj., showing PM (s) from a PMA (TEM, ×9720).
14. Head kidney of juvenile after 14d of the 10th BSA inj., showing PM from a PMA (TEM, ×7830).

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鲇鱼成鱼和幼鱼中含色素的巨噬细胞集结的诱导发生

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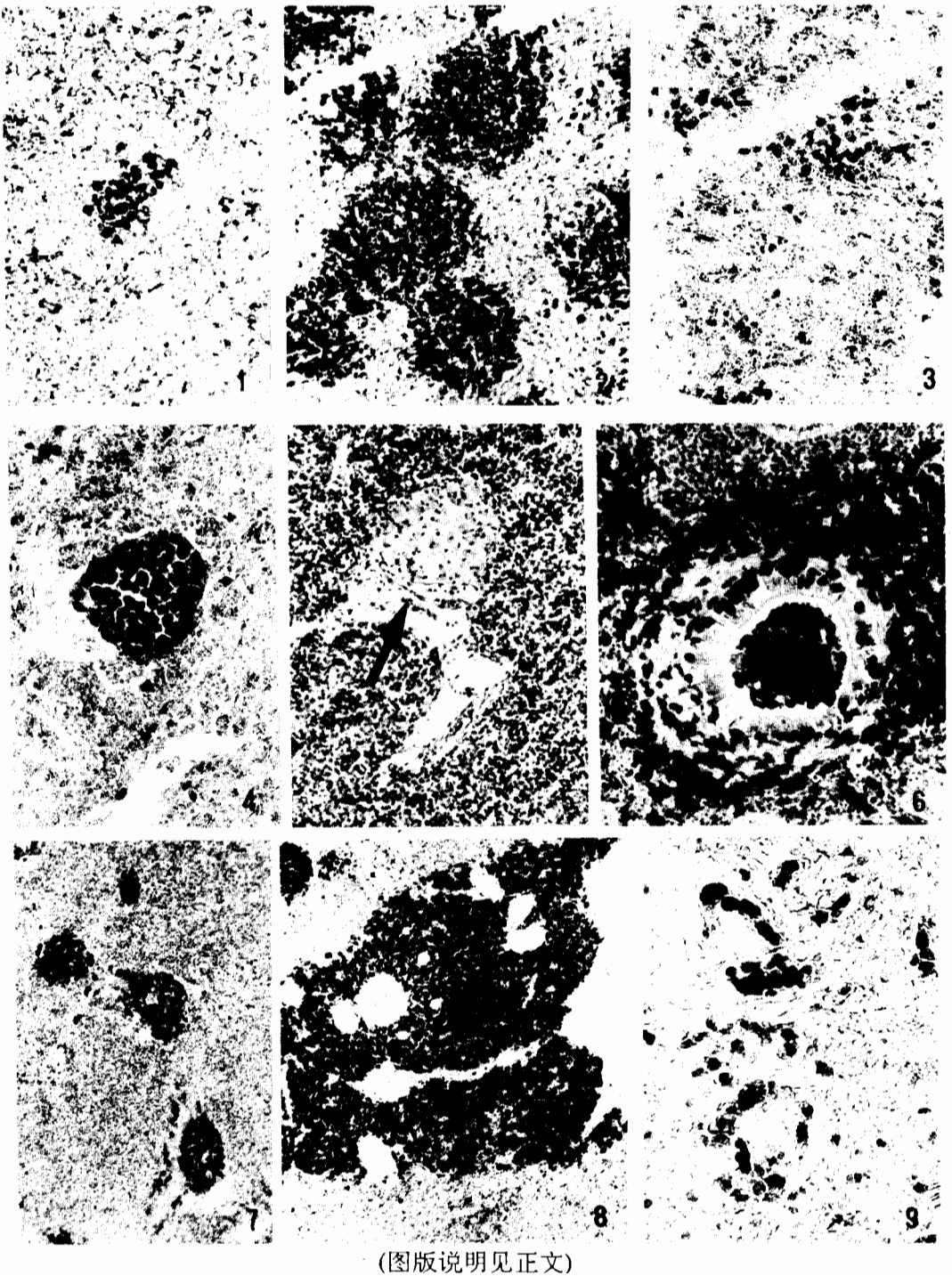
摘要　通过腹腔注射不同剂量的牛血清白蛋白[(BSA)或墨汁以刺激巨噬细胞活动，研究了鲇鱼成鱼和幼鱼头肾、肾、脾和肝中含色素的巨噬细胞集结(PMA)的发生。鲇鱼淋巴样组织中存在巨噬细胞的不均一性，可分网状／纤维状巨噬细胞，梭状巨噬细胞，圆形红色巨噬细胞，圆形黄红色巨噬细胞和含色素的巨噬细胞。注射 BSA 或墨汁后，头肾，肾，脾和肝中 PMA 和圆形巨噬细胞的数量最终均有不同程度的增加。通过诱导发生实验，表明含色素的巨噬细胞是由其他巨噬细胞衍变形成的。PMA 的形成与圆形巨噬细胞的总密度紧密相关：在圆形巨噬细胞的总密度较高时，容易形成 PMA 或巨噬细胞集结(MA)；在圆形巨噬细胞的总密度很低时，则不形成 PMA 或 MA。在连续多次注射 BSA 的幼鱼，饱含吞噬物的圆形巨噬细胞或 PMA 在实验后期则向外迁移出淋巴样组织，从而使 PMA 和圆形巨噬细胞的密度逐渐降低。根据实验结果，对 PMA 的功能进行了讨论并提出了作者的观点。

关键词　含色素的巨噬细胞集结，发生，鲇鱼

Zhong Mingchao *et al.*: Induced development of pigment macrophage aggregate(PMA) in adult and juvenile of the freshwater catfish *Silurus asotus* L.



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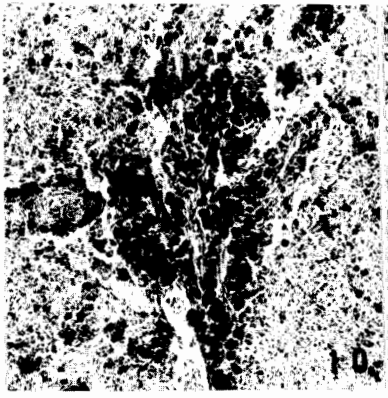

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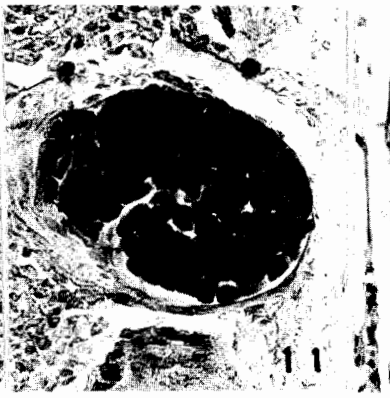

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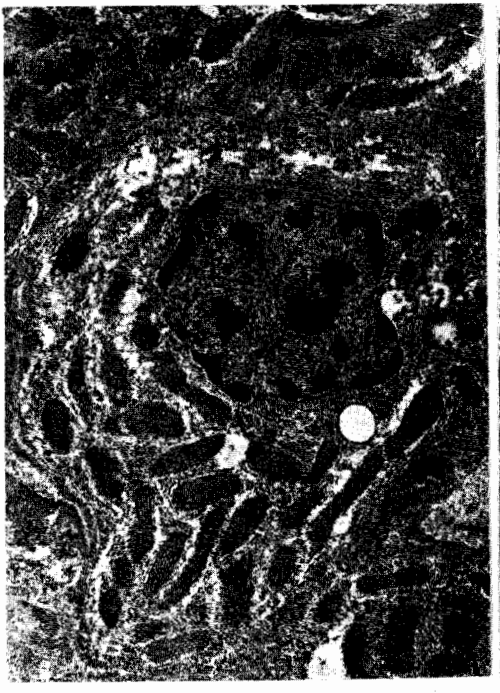

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